REMARKS

The claims and specification have been amended to correct minor informalities. Claim 3 has been cancelled. Claims 36-38 have been added. Claim 1 has been amended to more clearly claim the invention. Claims 5-9 have been amended to be multiply dependent on either Claim 1 or Claim 36. Support for the amendments to Claim 1 and for new Claims 36 and 37 can be found in the Specification and Claims as filed, for example, Claims 1, 2, 3 and 10. Support for Claim 38 can be found, e.g., at Example 4 on page 27. In addition, Claims 16-35 have been cancelled solely as drawn to a non-elected invention.

The changes made to the specification and claims by the current amendment, including ~~deletions~~ and additions, are shown herein with deletions designated with a strikethrough and additions underlined. No new matter has been added herewith. As a result of the Amendment, Claims 1, 4-15 and 36-37 are presented for further examination.

IDS

The IDS is enclosed herewith as required by CFR 1.98(b).

Specification objections

The Specification has been amended as requested by the Examiner to define abbreviations and to spell them out when appropriate.

Claim Objections

The Examiner objected to Claims 2, 10 and 4 for the following informalities. Claims 2 and 10 recited "EPL and ES" but have been amended to provide the full name of the abbreviations. Claim 4 contained a spelling error: "bane" which has been amended to read "bone". The Claims have been amended as suggested by the Examiner. In view of the amendments, Applicants respectfully request withdrawal of the claim objections.

Rejections under 35 U.S.C.§112, first paragraph – Written Description

The Examiner has rejected Claims 1-15 as failing to comply with the written description requirement. More specifically, the Examiner believes that the types of cells were defined quite

broadly. However, Claim 1 now recites "A method for directing a population of embryonic stem cells." Thus, withdrawal of this aspect of the rejection is respectfully requested.

The Examiner referred to Claim 4, which has now been amended to depend from Claim 36. Claim 4 relates to a number of cell types that are cells of mesodermal lineage. Enclosed in Appendix A are three publications which clearly demonstrate an effect of BMP4 on a variety of cells including embryonic stem cells (Rathjen, et al. J. of Cell Science 112:601-612, 1999; Smith et al. Dev. Biol 151:339-351, 1992; and Lake et al. J. of Cell Science 113, Pt3:555-566, 2000). None of the prior art references, however, demonstrates the passage of cells in the presence of BMP4 down the mesodermal pathway. However, it is clear that many cells do react to BMP4. The essence of the invention is the conditions used to differentiate ES cells ultimately down the mesodermal pathway or to direct cells of mesodermal lineage to mesodermal tissue. Consequently, we disagree with the Examiner's allegation that the claims are too broad. The references demonstrate support for an effect of BMP4 on a range of stem cells.

The Examiner further states that there is insufficient written description for the reference in Claim 1 to a "homologue, analogue or functional equivalent thereof" of BMP4. The claims has been amended to remove reference to "homologue and analogue" and to specify that the "functional equivalent thereof" directs said cells to preferentially differentiate into mesodermal tissue.

The Examiner further states that there is insufficient written description for "MEDII or its functional equivalent" in Claim 10. The Examiner believes that it is unclear what constitutes a "functional equivalent" of MEDII. MEDII is a very well characterized medium based from HepG2 cells. There are many articles on MEDII which can be identified by doing any type of internet search, even as broad as a Google search. These articles identify two main active ingredients: the amino acid L-proline and the protein fibronectin. One such article is by the inventors published in Biology of Reproduction 69:1863-1871, 2003. Thus, any media like DMEM which is conditioned by HepG2 or an equivalent cell can be used. .

Claims 1-9 were believed deficient in written description for a method of directing a population of any types of cells along a mesodermal cell lineage. The Examiner believes that the specification only contains written description for directing a population of undifferentiated cells along a mesodermal lineage. There appears to be some confusion as to the process undertaken in generating cells to move down the mesodermal lineage. In essence, the method of the present

invention is the culturing of ES cells in a medium such as MEDII so that they partially differentiate into EPL cells which, in a suspension culture, form EPL embryoid bodies. In the presence of BMP4, the cells in the embryoid bodies are then directed along a mesodermal lineage. Additionally, cells already proceeding down a mesodermal pathway in the presence of BMP4 differentiate into mesodermal tissue. The claim has been amended to read that ES cells are preferentially differentiated into mesodermal tissue. As such, the written description for such claims appears to be clearly established.

Rejections under 35 U.S.C.§112, second paragraph

The Examiner has rejected Claims 1-15 as indefinite for the following reasons:

Claim 1 recites that the cells are cultured in the presence of BMP4 "for a time and under conditions sufficient for said cells to preferentially differentiate into mesodermal cells". The examiner does not believe that the time and conditions are definite and further believes that the word "preferentially" is indefinite. The claim has been amended to remove the terms "preferentially" and "conditions" thus rendering the claim definite.

Claim 10 is believed indefinite for the recitation "culturing ES cells or EPL cells in MEDII". The Examiner believes that it is not clear how ES or EPL cells can be cultured if EPL cells are formed by culturing ES cells in MEDII. Applicants respectfully believe the rejection is inappropriate when it is clear that even when ES cells have been formed by culture, they require extra time for aggregation as part of the differentiation process. Thus, the statement is believed definite.

Rejections under 35 U.S.C.§102(b)

The Examiner has rejected Claims 1, 3-5, and 7-9 as anticipated by Schuldiner et al. (PNAS, 2000), in light of R&D Systems, in light of information from Dr. Benvenisty (of Schuldiner et al.). More specifically, the Examiner believes that Schuldiner et al describes a method of culturing human ES cells (H9 clone) in the presence of BMP4 (p. 11307, col. 2-p. 11308). Since the cells differentiated into chondrocytes and blood cells and since the species origin was identified by the Examiner to be human and the BMP4 was identified to be human, the Examiner believes that the claims are anticipated by Schuldiner et al.

To be anticipatory under 35 U.S.C. § 102, a reference must teach each and every element of the claimed invention. *See Hybritech Inc. v. Monoclonal*

> *Antibodies, Inc.*, 802 F.2d 1367, 1379 (Fed. Cir. 1986). "Invalidity for anticipation requires that all of the elements and limitations of the claim are found within a single prior art reference. ...There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention." *See Scripps Clinic & Research Foundation v. Genentech, Inc.*, 927 F.2d 1565 (Fed. Cir. 1991).

Schuldiner et al. does not include a step in which the ES cells are first cultured to form earlier primitive ectoderm-like (EPL) cells. In contrast, the amended claims specify that the ES cells are first cultured to form EPL cells. The EPL cells are then cultured to form embryoid bodies (EB). The cells in the embryoid bodies in the presence of BMP4 then produce mesoderm tissue.

Therefore Schuldiner et al. does not anticipated the claimed invention because Schuldiner et al. does not teach or suggest a step in which the ES cells are first cultured to form earlier primitive ectoderm-like (EPL) cells.

Since the Schuldiner et al. reference teaches the treatment of ES cells directly with BMP4, it also does not anticipate Claim 36, which requires treatment of "cells of mesodermal lineage." Thus, Schuldiner et al. does not anticipate any of the presently pending claims.

Rejections under 35 U.S.C.§102(b)

The Examiner has rejected Claims 1, 3-4, and 7-8 as anticipated by Finley et al. (J. Neurobiology, 1999). More specifically, the Examiner believes that Finley et al teaches culturing mouse ES cells in the presence of BMP4 (p. 273, col 1-2) which differentiated into cells of a mesodermal lineage (evidenced by morphology, presence of vimentin, and increase in expression of *brachyury*) and thus anticipates the claimed invention.

Finley et al. does not include a step in which the ES cells are first cultured to form earlier primitive ectoderm-like (EPL) cells. In contrast, the amended claims specify that the ES cells are first cultured to form EPL cells. The EPL cells are then cultured to form embryoid bodies (EB). The cells in the embryoid bodies in the presence of BMP4 then produce mesoderm tissue.

Therefore Finley et al. does not anticipated the claimed invention because Finley et al. does not teach or suggest a step in which the ES cells are first cultured to form earlier primitive ectoderm-like (EPL) cells.

Since the Finleyr et al. reference teaches the treatment of ES cells directly with BMP4, it also does not anticipate Claim 36, which requires treatment of "cells of mesodermal lineage." Thus, Finley et al. does not anticipate any of the presently pending claims.

Rejections under 35 U.S.C.§102(b)

The Examiner has rejected Claims 1, 3-4, and 6-8 as anticipated by Johansson et al. (Mol. Cell. Biol., 1995), in light of Stem Cell Technologies, Inc. More specifically, the Examiner believes that Johansson et al. teaches culturing Mouse ES cells in the presence of human BMP4 (p. 142, col. 1, p. 145, col. 5) and that the ES cells differentiated into mesodermal cells by day 5 (cardiomyocytes and haematopoietic precursors).

Johannson et al. does not include a step in which the ES cells are first cultured to form earlier primitive ectoderm-like (EPL) cells. In contrast, the amended claims specify that the ES cells are first cultured to form EPL cells. The EPL cells are then cultured to form embryoid bodies (EB). The cells in the embryoid bodies in the presence of BMP4 then produce mesoderm tissue.

Therefore Johansson et al. does not anticipated the claimed invention because Johansson et al. does not teach or suggest a step in which the ES cells are first cultured to form earlier primitive ectoderm-like (EPL) cells.

Since the Johannson et al. reference teaches the treatment of ES cells directly with BMP4, it also does not anticipate Claim 36, which requires treatment of "cells of mesodermal lineage." Thus, Johansson et al. does not anticipate any of the presently pending claims.

Rejections under 35 U.S.C.§102(b) and §103(a)

The Examiner has rejected Claims 5 and 6 under 35 U.S.C.§102(b) as anticipated by or in the alternative under 35 U.S.C.§103 as obvious in view of Finley et al. More specifically, the Examiner believes that Finley et al teaches culturing mouse ES cells in the presence of BMP4 (p. 273, col 1-2) which differentiated into cells of a mesodermal lineage (evidenced by morphology, presence of vimentin, and increase in expression of *brachyury*) and thus anticipates the claimed invention. Claim 5 requires the cells be from a homologous species and Claim 6 requires they be from a heterologous species.

The law is clear that three basic criteria must be met to establish a *prima facie* case of obviousness: (MPEP ¶2143):

> First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references, when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure (*In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1440 (Fed. Cir. 1991)).

As discussed above in connection with the anticipation rejection, Finley et al. does not include a step in which the ES cells are first cultured to form earlier primitive ectoderm-like (EPL) cells. In contrast, the amended claims specify that the ES cells are first cultured to form EPL cells. The EPL cells are then cultured to form embryoid bodies (EB). The cells in the embryoid bodies in the presence of BMP4 then produce mesoderm tissue.

Therefore Finley et al. does not anticipate the claimed invention because Finley et al. does not teach or suggest a step in which the ES cells are first cultured to form earlier primitive ectoderm-like (EPL) cells.

With respect to the obviousness rejection, Finely et al does not teach or suggest all of the claimed limitations because Finely et al does not teach that the ES cells are first cultured to form earlier primitive ectoderm-like (EPL) cells. Further, there is nothing in Finley et al to suggest that ES cells can be induced to form EPL cells, thus there is not suggestion to add this step to the method of Finley et al.

Rejections under 35 U.S.C.§103(a)

The Examiner has rejected Claims 1-4, 7-10, & 13-15 under 35 U.S.C.§103 as unpatentable over Lake et al (Journal of Cell Science, 2000) in view of Schuldiner et al (PNAS, 2000), Finley et al (J. Neurobiology, 1999), and Johansson et al (Mol. Cell. Biol. 1995).

The Examiner admits that Lake et al uses ES DMEM instead of DMEM conditioned with 50% MEDII during the aggregation of EPL cells to form embryoid bodies, and the transfer of aggregated EBs to gelatin-treated wells (See Lake et al. Pg. 556, col. 2; See Specification, Pg. 25-26). Lake cultures the cells in a suspension culture. Further, Lake et al does not teach that when BMP4 is added the ES or EPL differentiate into mesodermal cells. The Examiner suggests that the other references cited teach that BMP4 increases the degree of differentiation into mesodermal cells.

However, one of ordinary skill in the art would not have had a reasonable expectation of success in combining Lake et al. with any of the other references. There is nothing in Lake et al to suggest the addition of BMP4 would increase the differentiation of EBs into mesodermal cells. In Lake, even without the use of additional agents, 40-60% of the EBs differentiated into mesodermal cells (in the form of cardiomyocytes).

Johansson et al. teaches that when EBs were grown without BMP4 no cardiomyocytes are formed. When BMP2 is added, 20-40% of the EBs differentiated into cardiomyocytes. Thus, these references taken together would suggest to the skilled artisan that the addition of BMP4 might result in a reduced amount of mesodermal cells.

Schuldiner et al. show that without growth factors the cells differentiate into many different types of colonies. With BMP-4, the cells differentiated into large round cells (see page 11309, column 1, 3rd paragraph in Results). Thus, Schuldiner does not suggest that the addition of BMP-4 would even result in the production of mesodermal tissue.

Finley et al. show an increase in mesodermal differentiation when EBs are exposed to BMP-4 (page 284, column 1). However, no quantitative data is provided.

Thus, the three secondary references suggest that exposure of EBs to BMP-4 can lead to at best, a moderate increase in the percentage of mesodermal cells produced from EBs, along the lines of the 20-40% shown in the Johansson et al. reference. However, the Lake et al. reference discloses that 40-60% of the EBs can be converted to mesodermal cells even in the absence of BMP-4. Thus, if anything the secondary references suggest that the percentage of cells differentiated into mesodermal tissue would be decreased by the addition of BMP4. Thus, there is no motivation to combine the references to produce the claimed invention.

Moreover, and surprisingly, the present inventors discovered that 72.9% of EB aggregates formed cardiomyocytes when cultured with both MEDII and BMP4. *See,* Example 4 on page 27 of the specification. Nothing in the prior art suggests that such a high differentiation rate could have been achieved. In view of the results of the prior art, one skilled in the art would not have reason to successfully produce the claimed invention. Furthermore, these surprising results further evidence the nonobviousness of the claimed invention.

In view of the arguments and amendments herein, Applicants respectfully request withdrawal of the rejection under 35 U.S.C.§103(a).

Conclusion

Applicants believe that the current amendments place the application in condition for examination. Should there be any questions which might result in a delay in examination, the examiner is respectfully requested to contact the undersigned at the telephone number appearing below. Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated: April 21, 2005

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